Conditional and region-specific expression of transgenes in the brain using knock-in mice, the CRE/loxP system and adenoviral vectors

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Published in:
Journal of Neurochemistry

Publication date:
2001

Young Investigators' Colloquia 1: Advances in the gene transfer techniques aiming at gene therapy of the nervous systems

C01-01

Lentiviral vectors

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Gene therapy approaches rely on efficient gene transfer to the desired target cells. Although retroviral vectors have been the most widely used for gene therapy applications, the requirement of cell division for viral genome integration limits their use in nondividing target cells. In contrast, lentiviruses such as HIV-1 can infect both dividing and nondividing cells. Taking advantage of this feature, we have developed a lentiviral vector based on HIV-1. We have shown that lentiviral vectors pseudotyped with VSV-G can mediate efficient and stable transduction of postmitotic cells in brain, liver, muscle, and retina. Furthermore, lentiviral vectors were able to efficiently transduce human and mouse hematopoietic stem cells without cytokine prestimulation. Recent studies demonstrated the possibility of eliminating all accessory genes (*vif, vpr, vpu,* and *nef*) and *tat* from a packaging construct without losing the ability to transduce most nondividing cells. To further increase safety, we have developed self-inactivating (SIN) vectors in which the promoter and enhancer sequences in the LTR have been deleted. The potential of lentiviral vectors for gene therapy applications was tested in the *rd* mouse which is an animal model for retinitis pigmentosa (RP). RP is the most common inherited retinal disease in which photoreceptor cells degenerate, leading to blindness. Mutations in the rod photoreceptor cGMP phosphodiesterase β subunit (PDEβ) gene are found in patients with autosomal recessive RP as well as in *rd* mice. Injection of lentiviral vectors containing the PDEβ gene into the subretinal space of *rd* mouse eyes resulted in long-term rescue from photoreceptor degeneration. Lentiviral vectors promise great utility for gene therapy.

C01-02

Ribozymes for investigating gene function and gene therapy in the CNS

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The complexity of the CNS provides a great challenge for determining the physiological and pathophysiological functions of its molecular components. Ribozymes are catalytic RNA molecules that can be engineered to bind target RNA in an antisense-like specific manner allowing cleavage or editing of the target sequence. Consequently these molecules are being investigated in the nervous system as strategies for gene down-regulation and gene repair. The hammerhead ribozyme, one of the smallest identified, can yield site-specific RNA cleavage, and this ability to down-regulate gene expression makes it an ideal candidate for gene therapy or gene function analysis. In the gene therapy context this could be particularly useful for dominant genetic diseases by down-regulating the expression of mutant alleles, for example mutant forms of the huntingtin or α-synuclein genes linked to Huntington's disease and familial Parkinson's disease, respectively. With appropriate delivery systems, such as viral vectors, cell-specific down-regulation of genes can be achieved as a strategy to elucidate gene function. The function of the SMN gene in motor neurons and the FMR2 gene, underlying fragile X mental retardation, are currently being investigated. A second ribozyme motif, the group I intron ribozyme, is capable of site-specific RNA *trans*-splicing and it therefore has potential for RNA repair, for example, in the repair of trinucleotide-repeat mutant mRNA molecules such as those found in the DMPK gene in myotonic dystrophy or the huntingtin gene in Huntington's disease. Ribozymes are promising agents and should, in the future, play an important role in strategies for the therapy of neurological genetic disease and for investigating nervous system gene function.

C01-03

Conditional and region-specific expression of transgenes in the brain using knock-in mice, the CRE/loxP system and adenoviral vectors

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Immune cytokines have been shown to be important in neurodegeneration. Since most neurodegenerative processes are long-term events, to study the functional role of cytokines on neurodegeneration *in vivo* we tried to achieve chronic expression of cytokines in the brain. Transgenes were expressed for at least 2 months after inoculation of replication-deficient adenoviral vectors (Adv) in different brain regions. No overt signs of inflammation were observed when control Adv were inoculated. A dramatic effect on cell recruitment and survival was observed when Adv expressing cytokines were administered. In addition, we have generated knock-in mice in which a Tumor Necrosis Factor alpha (TNF) transgene is under the control of the endogenous engrailed promoter. In these mice, TNF expression is inhibited by the addition of stuffer sequences that are flanked by loxP sequences. The flanked sequence can be deleted by the Cre recombinase (CRE), allowing expression of the transgene. DNA recombination and substantia nigra-specific TNF expression was seen in AdCRE-injected knock-in mice but not in knock-in mice injected with control Adv or in AdCRE-injected control animals. This combination of techniques that allow temporal regulation of gene expression should provide the means to establish new animal models of disease as well as facilitate the study of chronic pathophysiology in the adult mouse brain.

C01-04

Adult mammalian bone marrow gives rise to cells that express neural antigens

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Bone marrow stem cells develop into a variety of hematopoietic and stromal lineages and repopulate the immune system throughout adult life. Recently, we found that after intraperitoneal transplantation, mouse bone marrow cells migrate into the brain and differentiate into neurons. In this study, bone marrow cells from male donors were transplanted into female PU.1 knockout mice, which lack mature immune cells. In the absence of marrow transplants, homozygous mice died within 48 h after birth. However, transplant recipients developed normally. Male donor cells were identified and characterized in female recipients by the presence of the Y-chromosome and cell type-specific antigens. After 1 month, donor cells were present in germinal regions and throughout the brain and 2% of all donor cells expressed the neuronal antigen NeuN. Acutely isolated stromal stem cells cultured in the presence of platelet-derived growth factor become nestin positive. Moreover, under the appropriate conditions, bone marrow stem cells differentiate into oligodendrocytes, the myelinating cells of the CNS. We transplanted 'tagged' (LacZ or GFP) bone marrow (hematopoeitic or stromal) or nestin positive stem cells into jimpy mice, which are characterized by dysmyelination and premature death. One month after receiving intraventricular injections, white matter contained donor cells that expressed oligodendrocyte-specific antigens, including myelin basic protein. Once the growth conditions needed to increase the number of bone marrow-derived neural cells are determined, they may provide a useful source of cells for gene therapy and the treatment of CNS disease and injury.

